DEVELOPMENT OF CAPRIC ACID FOR THERAPEUTIC MANAGEMENT OF AUTISM

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EXECUTIVE SUMMARY

Introduction: Autism or autism spectrum disorder (ASD) is defined as a heterogeneous neurodevelopmental disorder that is demonstrated by early-onset difficulties in social communication and behavior. They are characterized by unusually restricted, repetitive behavior and interests generally detectable by age 3. Apart from these core symptoms, patients also display a complex set of co-morbidities including cognitive deficits, anxiety, hyperactivity, aggression, and epilepsy. Important pathophysiological changes frequently described in the population comprise abnormalities in the normal metabolism, gut, immune, and mitochondrial functioning. The etiology of ASD is ascribed to both genetic and environmental factors that implicate several brain systems particularly gamma-aminobutyric acid (GABA), serotonergic, and glutamatergic systems. There is therefore a need for an optimized pharmacological treatment strategy for the management of autism that would target the abnormalities related to the above systems. Among the various interventions, nutritional interventions are considered a potential therapeutic option for such populations as these special diets seem to play a certain role in managing neurodevelopmental disorders. A medium-chain triglyceride (MCT) ketogenic diet comprising medium-chain fats improves core features of autism spectrum disorder in an open-label clinical trial in children aged 2 to 17 years old and warrants further investigation. Capric acid (CA), also referred to as decanoic acid, is a medium-chain fatty acid that forms one of the important components of a medium-chain triglyceride ketogenic diet. Reports suggest that CA has actions such as overpowering α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor (AMPA) receptor, diacylglycerol kinase, phosphoinositide signaling, mTORC1 pathway, stimulation/activation of peroxisome proliferator-activated receptor gamma (PPAR-γ), modulation/control of neuronal excitability mediated via several voltage-gated ion channels and neurotransmitters and hence pose to be a beneficial option on neuropsychiatric disorders. However, there is a lack of scientific evidence that confirm its safety and toxicity profile. N_2a, a mouse neuroblastoma cell line, due to its ability to differentiate and change shape, this cell line has proven to be significant in the testing of some neurotoxic medicines and chemicals with potential use in cytotoxic drugs. Here, glutamate which is an excitatory neurotransmitter, can accumulate in the brain and induce toxicity to the cell line when its

concentration is abnormally high, through oxidative damage. Similarly, test guidelines of OECD 423 help to determine the therapeutic doses that use a least number of animals per step to define the *in vivo* effects of the drug. Even though the research involving rodents and primates is on the surge, there is still a paucity of well-defined animal models for autism which necessitates the need for creating models that would be parallel to humans. Based on the emerging concept of gut dysbiosis in autism, an animal model was conceptualized in which common broad-spectrum antibiotics were used to create gut dysbiosis, and its effects on the behavior of pups were analyzed. At the same time, capric acid was also validated through a well-established valproic acid-induced model and antibiotic-induced model.

Aim/Objective: The study targets to determine the safety and toxicity profile of the fatty acid by *in-vitro* cell line and acute toxicity studies. The doses confirmed from the preliminary study were evaluated in a well-established valproic acid model of autism and a perinatal antibiotic exposure model parallel to human disorder.

Methods: In this study, the varied effects of CA were assessed *in vitro* by both Glutamate (10mM) and LPS (1µg/ml) induced toxicity in the N2a cell line. The potential effect of CA was evaluated on cell viability, oxidative stress, mitochondrial membrane potential, and inflammatory parameters. The acute toxicity study included female albino Wistar rats (3 per step) which were administered with a starting oral dose of 300 mg/kg and 2000 mg/kg respectively for 14 days. They were observed for signs of toxicity specifically for the first 4 hours, 24 hours, and then consecutively up to the next 14 days. Signs of lacrimation, salivation, piloerection, drowsiness, tremors, convulsions, diarrhea, and changes in fur, eyes, and mucous membranes were noted. Periodic evaluation of weight, food/water intake, and behavioral changes were also done on days 0, 7, and 14. Biochemical and hematological changes were evaluated after the study on the 14^{th} day. *In-vivo* effect of CA was studied in a single dose administered Valproic acid (VPA) (600mg/kg, *i.p.*) and Penicillin (31mg/kg, *p.o.*) exposed animals (during the perinatal period) by evaluating their behavioral and biochemical parameters. The establishment of the disease condition was validated by checking out for developmental impairment in valproic acid-induced pups whereas 16sr RNA sequencing was done for affirmation of gut dysbiosis in penicillin-induced pups. Tests that assessed behavioral parameters, including stereotypical and

repetitive behavior, anxiety, depression, and levels of social interaction, learning/memory, and other confounding variables such as thermal nociception, motor coordination, and locomotor activity, were performed. On the biochemical front, parameters assessing oxidative stress, levels of pro-inflammatory cytokines, activities of mitochondrial complexes, as well as neurotransmitter levels in the designated brain regions were investigated following the behavioral tests. Histopathological analysis of the brain regions was also performed to assess the particular changes induced by valproic acid in the brains of the exposed rats.

Results: In the cell line study, capric acid at several doses (5, 10, 25, and 50μM) improved the changes induced by glutamate and LPS in cell morphology and reinstated the oxidative stress markers, cytokines levels, and loss of mitochondrial membrane integrity. At the same time, capric acid at 100μM displayed toxicity analogous to glutamate and LPS control in MTT and LDH assays.

In the acute toxicity/OECD 423 study, the oral dose of capric acid was found to be nontoxic up to 2000 mg/kg body weight in rats. No substantial signs of toxicity, body weight/behavioral/biochemical/hematological changes, or mortality were noted. The histopathological observations also ruled out any pathological changes in the vital organs of animals that were treated with diverse doses of capric acid. Hence, the therapeutic doses of 100 mg/kg, 200mg/kg, and 400 mg/kg body weight were carefully chosen for the subsequent experimental models.

Based on the *in vivo* results, it was observed that valproic acid successfully produced autism-like symptoms in the pups right from the PND 7 where development in them was very much impaired. The pups also demonstrated a multi-symptomatic behavior where most of the conditions like impaired social behavior, learning and memory, and anxiety and depression coexisted. VPA was found to produce oxidative stress, neuro-inflammation, and change in glutamate neurotransmitter levels in the hippocampus, prefrontal cortex, and cerebellum. The detrimental effect of VPA was also confirmed by histopathological sections of the selected brain regions. Similarly, administration of Penicillin during the perinatal period (critical period of development) resulted in gut dysbiosis of pups which produced long-term changes in behavioral (multi-symptomatic behavior) and biochemical (oxidative stress and neuro-inflammation) patterns resembling clinical symptoms of

autism. Treatment with capric acid produced a positive effect on the alterations with maximum effects evident at 400 mg/kg, *p.o.* through amelioration of behavioral as well as biochemical changes.

Conclusion: The *in-vitro* cell line and acute toxicity studies concluded that capric acid is safe to administer and thus can be advocated to be a therapeutic option in diseases. Administration of agents such as valproic acid and antibiotics like penicillin during a critical period of development of pups produced long-lasting changes in behavior and biochemical values symbolizing the condition of autism and capric acid can be considered as a potential candidate for the treatment and management of autism.

Treatment with capric acid significantly reduced the effects of VPA and Penicillin on stereotypical and repetitive behavior, social behavior, anxiety and depression-like state, learning and memory, and other confounding variables mainly through its broad neuroprotective effects via significant modulation of neurobehavioral parameters, oxidative stress, and inflammatory markers.